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Title of the Invention: Food Preservation Method

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DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for preservation and quality improvement of foods. More specifically, it relates to a method for preservation and quality improvement of foods which is characterized by adding oxidoreduction enzymes and bacteriolytic enzymes to foods.

The oxidoreduction enzymes according to the present invention are oxidoreduction enzymes which have oxygen as a receptor, and examples of which include glycolate oxidase, lactate oxidase, glucose oxidase, hexose oxidase, galactose oxidase, aldehyde oxidase, xanthin oxidase, pyruvate oxidase, oxalate oxidase, D-aspartate oxidase, L-amino acid oxidase, D-amino acid oxidase and amine oxidase. Also, bacteriolytic enzymes are, for example, lysozyme (E.C. 3.1.17) or lysozyme salts, enzymes produced by *Bacillus subtilis*, enzymes produced by *Streptomyces griseovilens*, and enzymes produced by *Brevibacterium lyticum*.

The oxidoreduction enzymes used according to the invention are enzymes which react with their substrates to

produce hydrogen peroxide, and they are extracted and purified from animal organs, plants and microorganisms.

For example, hexose oxidase is prepared from unripe oranges, and glucose oxidase is prepared from molds of the genus *Penicillium*. L-amino acid oxidase and D-amino acid oxidase are obtained from microorganisms.

Of the bacteriolytic enzymes used for the present invention, lysozyme is an enzyme which hydrolyzes the ß(1-4) bond of mucopolysaccharides and mucopolypeptides, and is widely distributed in the animal and plant kingdoms while also being produced by certain microorganisms, although practical supplies of lysozyme originate mainly from egg albumin. Egg albumin lysozyme is a single-chain polypeptide with a molecular weight of approximately 14,500 and an isoelectric point of 10.5-11.0, having a characteristic sweet taste.

Foods whose storage is particularly difficult, such as meat products, kneaded aquatic products, custard cream, soft cream and the like, have problems associated with their preservation which still cannot be overcome with the currently used preservatives alone, even when the use of such preservatives is permitted. Even more difficult is the storage of such foods as milk, raw noodles, cream puffs, for which the use of preservatives is not permitted, and these have become troublesome products for manufacturers and distributors. It has therefore been sought to establish a preservation method which is non-toxic and highly safe.

The present inventors have conducted diligent research in the light of these circumstances, resulting in the finding that this desired object may be achieved by the combined use of oxidoreduction enzymes and bacteriolytic enzymes.

Specifically, the present invention has been completed upon the discovery that a more notable effect is achieved by the use of oxidoreduction enzymes in tandem with bacteriolytic enzymes, instead of alone.

It is therefore an object of the present invention to provide a novel method for preservation and quality improvement of foods.

The invention may be applied to all types of foods, and specific examples thereof include meat products, kneaded aquatic products, milk, butter, cheese, soft drinks, juices, raw noodles, unsweetened bean jam, custard cream, fresh cream, butter cream, ice cream, soft cream, and the like.

The enzymes may be added by conventional methods for the purposes of the present invention. The amount of addition of each is not particularly restricted, but is suitable at about 5-500 ppm.

The active mechanism of the preservation effect of the present invention in foods is not completely understood, but is believed to result from reaction between the oxidoreduction enzyme and its substrate and subsequent elimination of microorganisms in the foods by the hydrogen peroxide by-product, as well as the reduction of oxygen in

the food, which suppresses growth of aerobic microorganisms.

The antibacterial effect of this hydrogen peroxide by-product is also much more powerful than that of hydrogen peroxide presently used for bleaching and sterilization of foods.

The effect of the invention will now be explained by way of experimental examples.

Experimental Examples

Different types of bacteria were seeded into trypto soy broth (pH 7.0) with lysozyme concentrations of 0-40 ppm and different types and amounts of oxidoreduction enzymes, and then cultured at 30°C for 4 days, after which the state of growth of the bacteria was visually observed. The results are shown in Tables 1 to 3.

Table 1 *E. Coli*

		L-amino acid oxidase (ppm)			
		0	5	25	125
Egg albumin lysozyme (ppm)	0	++	++	++	++
	10	++	+	-	-
	20	++	+	-	-
	40	++	±	-	-

Table 2 *Bacillus subtilis*

		Glucose oxidase (ppm)			
		0	10	20	40
Egg albumin lysozyme (ppm)	0	++	++	+	+
	10	+	-	-	-
	20	+	-	-	-
	40	-	-	-	-

Table 3 *Staphylococcus aureus 209P*

		Hexose oxidase (ppm)			
		0	5	25	125
Egg albumin lysozyme (ppm)	0	++	++	+	-
	10	++	+	-	-
	20	+	+	-	-
	40	+	-	-	-

As clearly shown in Tables 1 to 3, oxidoreduction enzymes and egg albumin lysozyme have a synergistic effect against 3 typical putrefactive bacteria.

Examples of the invention will now be presented.

Example 1

To commercially available milk there were added 20 ppm of bacteriolytic enzyme produced by *Bacillus subtilis* and 20 ppm of glucose oxidase, and the resulting preservation effect was compared against each enzyme used alone. The values in the following table are the total number of bacteria per milliliter of milk.

Table 4

	Days preserved			
	0	2	4	6
Not added	3×10^2	7×10^4	7×10^5	6×10^8
Bacteriolytic enzyme (20 ppm)	-	8×10^3	1×10^5	2×10^8
Glucose oxidase (20 ppm)	-	4×10^3	3×10^5	3×10^7
Bacteriolytic enzyme + glucose oxidase	-	7×10^2	6×10^3	9×10^4

Example 2 Fish paste

A total of 10 kg of fish meat dehydrated after exposure to water, including 3 kg of white-croaker, 3 kg of kinguchi,

3 kg of sea eel and 1 kg of angelfish was passed through a meat grinder, and then 500 mg of glycolate oxidase and 500 mg of egg albumin lysozyme was added and the mixture was then passed through a grinder to coarseness and thoroughly mixed. After further addition of 300 g of table salt, 500 g of sugar, 500 g of defatted soybean meal, 30 g of sodium glutamate and 200 ml of mirin, the mixture was subjected to further grinding, after which a mixed solution of 1.5 kg of starch and 3 l of water was added prior to additional thorough grinding.

The resulting ground fish meat was molded on a flat board and then steamed at 100°C for 15 minutes and cooled to obtain the product.

The fish paste obtained in this manner was placed in a thermostatic container and observed for stickiness, and while a glycolate oxidase-free group produced stickiness by the 3rd day, the added group exhibited stickiness only by the 10th day, and thus the storage life was improved by more than 2-fold. Upon addition of glycolate oxidase alone by the same method, stickiness was exhibited by the 8th day.

Example 3 Ball noodles

To 3.7 kg of wheat flour there were added 1.4 l of 15% saline, 450 mg of galactose oxidase and 200 mg of egg albumin lysozyme, and after mixing with a stirrer the mixture was kneaded. The kneaded product was then spread out with a roller, cut with a cutter, and then boiled in

boiling water to make balls of a prescribed amount.

The preservability of this product was considerably improved as evidenced by being edible after 60 hours at 30°C, compared to a preservative-free product which decayed within 24 hours to the point of being inedible. Also, a product prepared in exactly the same manner but with addition of only 450 mg of galactose oxidase became inedible due to decay within 48 hours. Thus, a very superior effect was obtained by the combined addition of both components.

Example 4 Custard cream

Custard cream was prepared according to the following recipe.

Glucose	100 g
Sugar	700
Wheat flour	100
Corn starch	100
Chicken eggs	30
Vanilla flavoring	q.s.
Milk	2 l
Hexose oxidase	100 mg
Egg albumin lysozyme	60 mg

This product and another product prepared without addition of hexose oxidase and egg albumin lysozyme were compared by organoleptic test and by bacteria count.

The results are shown in Table 5.

Table 3

Days passed	1	3	4	5	6
Control	-	+ 10^7	++ $2x10^8$		
Hexose oxidase + lysozyme	-	- $6x10^4$	- $6x10^5$	- $7x10^6$	± $8x10^7$

CLAIM

A method for preservation and quality improvement of a food, characterized by adding an oxidoreduction enzyme and a bacteriolytic enzyme to the food.